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DIVISION OF BACTERIOLOGY AND HYGIENE

STUDIES OF AGGLUTINATION REACTIONS IN HOG CHOLERA DURING THE PROCESS OF SERUM PRODUCTION

[Continued.]

BY
WARD GILTNER

EAST LANSING, MICHIGAN
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FOREWORD.

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The present bulletin continues the work published in Technical Bulletin No. 3 of this station, giving, however, more extensively and definitely the agglutinative relation of the blood-serum secured from normal, virus, immunized, and hyperimmunized pigs to *B. cholerae suis*. While the work established the fact that no dependence may be placed upon the degree of agglutination to indicate the potency of serum, it has been the means of providing many interesting and useful data as well as suggestions. These suggestions will be the basis of further investigations.

It becomes more and more apparent that, *notwithstanding the value and utility of the Dorset-Niles serum as it is now manufactured*, the problems concerning the practical manufacture and application call for renewed effort. A more intimate knowledge of the processes involved becomes essential to the most intelligent production and use. A solution can be found only in a study of the filterable virus, B. cholerae suis, and the organism of the pig as they are associated in this disease, hog cholera.

CHARLES E. MARSHALL.

STUDIES OF AGGLUTINATION REACTIONS IN HOG CHOLERA DURING THE PROCESS OF SERUM PRODUCTION.

(*Continued.*)

I. INTRODUCTION.

PURPOSE.

The purpose of this paper is to set forth the results of experiments made in an effort to complete the work previously published.[1] We are able to present a greater amount of data covering some of the points already considered and, in addition, a more or less comprehensive account of many features not previously considered.

A scientific understanding of swine epizoötics, covering such points as their etiology, serum therapy, and control, demands an answer to the preplexing question of the relation of *B. cholerae suis* (*B. suipestifer*) and *Bact. septicaemiae hemorrhagicae* (*Bact. suisepticum*) to these diseases. We have concerned ourselves only with the former of these organisms, the latter not having been isolated by us. As a result of our preliminary work, we were encouraged in the belief that a study of agglutination reactions in all the various stages of serum production might furnish a positive solution for some of the unsettled problems. We also hoped that there might be some definite relation between the agglutinative power of a serum toward B. cholerae suis and its immunizing power in vivo. In other words, we hoped to standardize the serum by the agglutination test.

PREVIOUS RESULTS.

Our work, in accord with that of many others, shows the pathogenicity of pure cultures of *B. cholerae suis* for swine under certain conditions. As a result of a limited number of observations, we have concluded that, "Pigs immunized according to the Turner-Kolle method may withstand intravenous injections of virulent cultures of *B. cholerae suis*." We are not aware of any one having demonstrated an outbreak of hog cholera due to *B. cholera suis* only,* excluding the possibility of the presence of the filterable virus as an etiological factor. A careful study of the literature added to our experience with hog cholera in connection with the production and application of Dorset-Niles serum leaves us undecided as to the necessity of opposing an artificial immunity against an organism regarded by many, as Hottinger[2] puts it, as a colon-like organism invading the blood from its habitat, the intestinal canal, and possessing only acquired pathogenic properties. What

* The work of Dammann and Stedefeder (Ref. 4) may furnish a possible exception to this statement.

the significance of "acquired pathogenic properties" may be, and what their acquisition may mean to a herd of hogs does not appear in the literature.

It seems to us good policy to persistently attempt to establish the fundamental points bearing upon the etiology of swine epizoötics, and, at the same time, vigorously but cautiously, to avail ourselves of all effective means of controlling these outbreaks even in the absence of a comprehensive understanding of the modus operandi of the means employed.

PLAN OF WORK.

The agglutination work has been conducted, as before, in connection with routine serum production, and on account of the great expense of this kind of animal experimentation, the plan has been to interfere as little as possible with the routine serum work. We have been able to follow the changes in the agglutinative power of the blood of a number of animals through various stages of treatment. The tabulated results show the differences in agglutinative power of the blood of normal pigs, of pigs treated with hog cholera virus alone or simultaneously with the protective serum, and of pigs treated with increasing doses of virus during the hyper-immunizing process. The results of efforts to measure the potency of serum by the agglutination reaction are shown under the tables covering the testing of mixed sera and table XXV.

TECHNIC.

The methods employed are essentially the same as in the previous work except that bouillon cultures of *B. cholerae suis* are used instead of a carbol-salt-solution suspension of agar slant cultures killed by heat. Flasks containing 50 cc. of ordinary bouillon are inoculated just before leaving the laboratory in the afternoon, and placed at 37°C. Upon returning to the laboratory 16 hours later, it is found that the cultures are uniformly turbid. A limited number of counts indicates the presence of from one-half to one billion organisms per cc. The cultures are too cloudy for the agglutination test, and are diluted by the addition of an equal volume of 0.85 per cent Nacl solution containing 0.4 per cent formalin. This puts the organisms in a 0.2 per cent formalin solution. The flasks are then kept in a cool room, and agitated daily. Sub-cultures usually show no growth after the second day.

The bacterial suspension prepared in this manner gives satisfactory results after 24 days at least, and probably can be used after a much longer time. Hiss,[3] working with dead typhoid cultures prepared in a similar way, states that there is "no change in their limit of agglutination even after weeks." The advantages in this method are the facility with which a large volume of bacterial emulsion is secured and the uniformity of the successive preparations.

It is essential in comparative agglutination tests that the number of organisms in the bacterial suspensions be fairly constant. The use of a nephelometer for standardizing the density of the emulsion does not appeal to us so strongly as uniform methods of producing the emulsion.

It has seemed desirable to determine in each case the limit of the agglutinative power of each serum tested. In order to do this, we have attempted to test the serum at dilutions both above and below the limit and at the same time to employ only five different dilutions. This is possible only when we can make a close estimate as to the probable maximums at which agglutination will occur. Previous tests with blood serum from the same animal and a knowledge of the treatment to which the animal has been subjected are the only indications. The agglutinative power of a serum, as measured by the system of dilutions, does not always diminish at a ratio capable of being computed from the data furnished by the observation of a considerable number of dilutions. To make this point clear, it is only necessary to study a table of reactions when it will be found that a complete reaction at one dilution is followed in some cases by a gradual diminution of the strength of the reaction through several succeeding dilutions while in other cases a complete reaction may be followed in unvarying intensity throughout a considerable number of dilutions. This discussion is of more general interest, however, than the subject of this paper will permit. Still, we are convinced that our understanding of the nature of the agglutination reaction and the technic involved in making the test is very crude.

CULTURES USED.

Two cultures from different sources, but probably the same strain, were used in all the tests recorded here. Comparative tests demonstrated that there was no appreciable difference in the agglutinability of these two cultures of *B. cholerae suis*. Indeed they must have had a common source unless the *B. cholerae suis* found in the blood and other tissues of "virus pigs" finds its way there from its alleged normal habitat, the intestines, of each pig treated.

Culture "virus 122" came from the spleen of virus pig 122.

Virus pig 122, wt. 75 lbs., was inoculated in the muscles of the ham 9-23-09 with 5 cc. virus blood from Expt. pig 216. The pig was killed 9 days after inoculation after showing characteristic symptoms of hog cholera.

Autopsy: Inguinal glands quite hemorrhagic; iliac glands enlarged and hemorrhagic; renal glands hemorrhagic; kidneys show few petechiae in both cortex and medulla; spleen enlarged and friable.

Culture "virus 136" came from the spleen of virus pig 136.

Virus pig 136, wt. 81 lbs., was inoculated in the muscles of the ham 11-30-09 with 5 cc. of virus blood from virus pig 128. Was killed 11 days later after showing symptoms of hog cholera.

Autopsy: Cortex of inguinal and iliac glands hemorrhagic; kidneys show petechiae in both cortex and medulla; spleen enlarged, dark and friable; liver shows slight superficial necrosis; intestinal mucosa sprinkled with numerous petechiae; lungs, hemorrhagic.

These cultures were plated on agar and typical colonies were seeded on the various media necessary for the identification of *B. cholerae suis*. Infectiousness for rabbits was also determined. Pathogenicity for pigs was not determined except that large quantities of culture "virus 136" were injected intramuscularly into an immune pig (Expt. 315) with the result that only local abscesses were produced.*

*The object and results of this work will be recorded later.

Since these cultures respond similarly when tested with the same sample of serum, it is of interest to trace their origin.

The original "Ames virus" which is the basis of all our routine work, was kept in sealed bulbs for over six months. The successive transfers were then as follows:

"Ames virus" to Expt. pig 124, to Expt. pig 199, to Expt. pig 216, to virus 122.

"Ames virus" to Expt. pig 123, to Expt. pig 197, to virus 124, to virus 128, to virus 136.

"In studying the reaction,—we take into consideration two factors, viz.—(a) The clumping or agglutination of the bacteria and consequent clearing of the test fluid and (b) The formation of a characteristic membrane on the bottom of the tube." In the tables under each dilution are the signs (+ — o) arranged in two columns to indicate the degree of reaction having reference to the two factors considered above.

TABLE I.—*Agglutination Tests With Normal Serum and Culture "Virus 122."*

In the table, the sign (+) indicates a complete reaction, the sign (—) indicates that the reaction *has progressed considerably, but not completely, and the sign (0) indicates no change.*

Dilution of serum.			25.		50.		100.		125.		250.		500.	
No. of pig.	Weight.	Date tested.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Expt. 293...	36 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 294...	33 lbs.	11-24-09	......	......	—	—	0	0	0	0	0	0	0	0
Expt. 295...	33 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 296...	27 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 297...	26 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 298...	26 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 299...	35 lbs.	11-24-09	......	......	—	—	0	0	0	0	0	0	0	0
Expt. 300...	42 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 301...	35 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 302...	32 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 303...	28 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 304...	30 lbs.	11-24-09	......	......	0	0	0	0	0	0	0	0	0	0
Expt. 305...	49 lbs.	12- 2-09	—	—	0	0	0	0	0	0	0	0	0	0
Expt. 306...	49 lbs.	12- 2-09	—	—	—	—	—	—	—	—	—	—	......	......
Expt. 307...	48 lbs.	12- 2-09	—	—	0	0	0	0	0	0	0	0	......	......
Expt. 308...	44 lbs.	12- 2-09	—	—	0	0	0	0	0	0	0	0	......	......
Expt. 309...	44 lbs.	12- 2-09	0	0	0	0	0	0	0	0	0	0	......	......
Expt. 310...	36 lbs.	12- 2-09	0	0	0	0	0	0	0	0	0	0	......	......
Expt. 311...	55 lbs.	12- 2-09	+	+	—	—	—	—	—	—	—	—	......	......
Expt. 312...	44 lbs.	12- 2-09	—	—	0	0	0	0	0	0	0	0	......	......
Expt. 313...	46 lbs.	12- 2-09	—	—	—	—	—	—	—	—	0	0	......	......
Expt. 314...	50 lbs.	12- 2-09	—	—	0	0	0	0	0	0	0	0	......	......
Expt. 315...	37 lbs.	12- 2-09	0	0	0	0	0	0	0	0	0	0	......	......
Expt. 316...	27 lbs.	12- 2-09	0	0	0	0	0	0	0	0	0	0	......	......
Expt. 317...	50 lbs.	12-18-09	—	—	0	0	0	0	0	0	0	0	......	......
Expt. 318...	51 lbs.	12-18-09	—	—	—	—	—	—	—	—	0	0	......	......
Expt. 319...	49 lbs.	12-18-09	0	0	0	0	0	0	0	0	0	0	......	......
Expt. 320...	44 lbs.	12-18-09	0	0	0	0	0	0	0	0	0	0	......	......
Expt. 221...	42 lbs.	12-18-09	—	—	—	—	0	0	0	0	0	0	......	......
Expt. 222...	53 lbs.	12-18-09	—	—	—	—	—	—	—	—	0	0	......	......
Virus 128...	96 lbs.	12-11-09	......	......	+	—	—	—	—	—	0	0	0	0
Virus 136...	83 lbs.	12-11-09	......	......	+	—	—	—	—	—	0	0	0	0

REACTIONS WITH NORMAL SERA.

We have already recorded the reactions with the blood of 9 normal pigs. Of these, 3 gave a reaction at 1-125, the remaining 6 gave only a slight reaction at 1-50.[1] In Table I are recorded the reactions with the blood of 32 normal* pigs. Of the 32 samples of normal blood tested,

6 or 18.75	per cent gave no reaction	at a dilution of	1—25.
6 or 18.75	per cent gave a reaction	at a dilution of	1—25.
10 or 31.25	per cent gave no reaction	at a dilution of	1—50.
3 or 9.375	per cent gave a reaction	at a dilution of	1—50.
5 or 15.625	per cent gave a reaction	at a dilution of	1—125.
2 or 6.25	per cent gave a reaction	at a dilution of	1—250.

In the second column of Table I are given the weights of the pigs at the time the sample of blood was drawn. It was found that, in the former records, of the three normal pigs whose blood gave a reaction at 1-125 that the average weight was 66 lbs., and of the six normal pigs whose blood gave a reaction at 1-50 the average weight was 23 lbs. We find from a study of Table I that of the

6 pigs giving no reaction	at 1—25	the average weight is	39 lbs.
6 pigs giving a reaction	at 1—25	the average weight is	47 lbs.
10 pigs giving no reaction	at 1—50	the average weight is	31 lbs.
3 pigs giving a reaction	at 1—50	the average weight is	37 lbs.
5 pigs giving a reaction	at 1—125	the average weight is	63 lbs.
2 pigs giving a reaction	at 1—250	the average weight is	52 lbs.

We see in these results a tendency for the agglutinative power of the serum to increase with the size or more probably the age of the pig. *This point cannot be considered determined by the few tests recorded* here. The results, however, are suggestive.

REACTIONS WITH THE BLOOD OF PIGS TREATED WITH VIRUS.

Table II shows the agglutination reactions with the blood of 56 pigs inoculated with virus of hog cholera, i. e., with blood drawn from a pig showing symptoms and lesions of hog cholera. There is also shown a considerable amount of other data included with the hope that the interpretation of the agglutination reactions may be facilitated.

*The word "normal" is used in the sense that the pig has neither been exposed to hog cholera nor subjected to any treatment.

TABLE II.—*Agglutination tests with the blood of pigs inoculated with virus.*

In the table, the sign (+) indicates a complete reaction, the (—) indicates that the reaction has progressed considerably, but not completely, and the sign (o) indicates no change.

Dilution of serum.							50.		100.		125.		250.		500.		800.	
Number of pig.	Weight, lbs.	Date sample drawn.	Amount of virus injected into pig.	Days lived after injection.	Died.	Killed.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Virus 121	75	10-2-09	5 cc. expt. 216	9	......	yes	......	......	......	......	......	......	......	......	—	—	0	0
Virus 122	75	10-2-09	5 cc. expt. 216	9	......	yes	......	......	......	......	......	......	......	......	—	—	0	0
Virus 12[illegible]	75	10-5-09	5 cc. expt. 197	10	......	yes	......	......	......	......	......	......	......	......	—	—	—	—
Virus 127	65	11-20-09	5 cc. virus 124	9	......	yes	—	—	0	0	0	0	0	0	0	0	......	......
Virus 128	63	11-20-09	5 cc. virus 124	9	......	yes	0	0	0	0	0	0	0	0	0	0	......	......
Virus 129	63	11-21-09	5 cc. virus 122	6	......	yes	+	—	+	—	+	—	+	—	—	—	......	......
Virus 130	52	11-22-09	5 cc. virus 122	7	......	yes	+	—	—	—	—	—	—	—	0	0	......	......
Virus 131	83	11-24-09	5 cc. virus 124	6	......	yes	+	+	—	—	—	—	0	0	0	0	......	......
Virus 132	91	11-26-09	5 cc. virus 124	7	......	yes	—	—	—	—	0	0	0	0	0	0	......	......
Virus 137	75	12-9-09	5 cc. virus 127	9	......	yes	+	+	+	+	+	+	+	—	—	—	......	......
Virus 138	96	12-15-09	5 cc. expt. 199	8	......	yes	+	+	+	—	+	—	+	—	—	—	......	......
Virus 140	84	12-22-09	5 cc. virus 136	8	......	yes	......	......	......	......	......	......	—	—	0	0	......	......
Virus 141	98	12-23-09	5 cc. virus 136	9	......	yes	......	......	......	......	......	......	—	—	—	—	......	......
Virus 143	83	1-7-10	5 cc. virus 124	8	......	yes	+	+	+	—	+	—	+	—	—	—	......	......
Virus 145	52	1-12-10	5 cc. virus 128	6	yes	......	......	......	......	......	......	......	+	—	0	0	0	0
Virus 146	51	1-12-10	5 cc. virus 128	6	......	yes	......	......	......	......	......	......	—	—	0	0	0	0
Virus 151	54	2-3-10	5 cc. virus 125	9	......	yes	—	—	0	0	0	0	......	......	......	......	......	......
Virus 152	54	2-3-10	5 cc. virus 127	9	yes	......	—	—	—	—	0	0	0	0	0	0	0	......
Virus 153	37	2-3-10	5 cc. virus 127	9	yes	......	—	—	—	—	—	—	0	0	0	0	......	......
Virus 156	63	2-9-10	5 cc. virus 107	7	......	yes	+	—	—	—	—	—	—	—	—	—	......	......
Virus 157	57	3-26-10	5 cc. virus 125	26	lived.	......	+	—	—	—	—	—	—	—	—	—	......	......
Virus 160	51	3-15-10	5 cc. virus 125	10	......	yes	—	—	—	—	—	—	—	—	0	0	......	......
Virus 161	43	3-17-10	5 cc. virus 125	9	......	yes	—	—	0	0	0	0	0	0	0	0	......	......
Virus 162	43	3-17-10	5 cc. virus 125	9	......	yes	0	0	0	0	0	0	0	0	0	0	......	......
Virus 165	55	3-25-10	8 cc. virus 121	8	......	yes	+	+	+	+	+	+	+	+	—	—	......	......
Virus 166	53	3-25-10	8 cc. virus 121	8	......	yes	—	—	—	—	—	—	—	—	—	—	......	......
Virus 167	61	4-16-10	10 cc. virus 106	8	......	yes	+	+	+	—	+	—	—	—	—	—	......	......
Virus 168	58	4-16-10	8 cc. expt. 199	8	......	yes	+	+	+	—	—	—	—	—	—	—	......	......
Virus 169	62	4-16-10	9 cc. expt. 268	8	......	yes	+	+	+	+	+	+	+	—	—	—	......	......
Virus 170	68	4-16-10	8 cc. expt. 197	8	yes	......	+	+	+	+	+	+	+	+	+	—	......	......

TABLE II.—*Concluded.*

Dilution of serum.							50.		100.		125.		250.		500.		800.	
Number of pig.	Weight, lbs.	Date sample drawn.	Amount of virus injected into pig.	Days lived after injection.	Died.	Killed.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Virus 171	60	4-16-10	9 cc. expt. 200	8	yes		+	—	—	—	—	—	—	—	—	—		
Virus 172	59	4-23-10	10 cc. virus 170	7		yes	—	—	—	—	—	—	—	—	0	0		
Virus 173	59	4-23-10	10 cc. virus 169	7		yes	—	—	—	—	—	—	—	—	0	0		
Virus 174	50	4-22-10	10 cc. virus 167	6		yes	—	—	—	—	—	—	—	—	0	0		
Virus 176	57	4-22-10	10 cc. virus 171	6		yes	+	—	—	—	—	—	—	—	0	0		
Virus 178	65	5-7-10	10 cc. virus 171	5		yes	+	+	+	+	+	—	—	—	—	—		
Virus 181	78	5-13-10	10 cc. expt. 398	8		yes	+	—	+	—	—	—	—	—	—	—		
Virus 183	124	5-21-10	10 cc. virus 179	5		yes	+	+	+	+	+	+	+	+	—	—	—	—
Virus 184	86	5-21-10	10 cc. virus 179	5		yes	+	+	+	+	+	+	+	+	—	—	—	—
Virus 185	92	5-26-10	10 cc. virus 182	7		yes	+	+	—	—	—	—	—	—	—	—		
Virus 186	75	5-26-10	10 cc. virus 182	7		yes	—	—	—	—	—	—	—	—	0	0		
Virus 187	138	6-16-10	10 cc. virus 125	7		yes	+	—	—	—	—	—	0	0	0	0		
Virus 188	125	6-16-10	5 cc. virus 175	7		yes	—	—	—	—	0	0	0	0	0	0		
Virus 189	106	6-21-10	10 cc. virus 170	7		yes	+	—	—	—	—	—	0	0	0	0		
Virus 190	111	6-21-10	8 cc. virus 175	7			+	—	—	—	—	—	0	0	0	0		
Virus 191	89	6-21-10	8 cc. expt. 425	7		yes	—	—	0	0	0	0	0	0	0	0		
Expt. 291	24	12-9-09	5 cc. from Canada	38	yes		—	—	—	—	—	—	—	—	0	0		
Expt. 298	25	12-26-09	1 cc. virus 124	8		yes	—	—	—	—	—	—	0	0	0	0		
Expt. 310	36	12-11-09	1 cc. virus 127	12	yes		+	+	+	+	—	—	—	—	—	—		
Expt. 316	27	12-11-09	1 cc. virus 128	11		yes	+	+	+	—	+	—	—	—	—	—		
Expt. 319	49	1-18-10	2 cc. virus 131 filtered	35	yes		0	0	0	0	0	0	0	0	0	0		
Expt. 377	13	1-31-10	1 cc. virus 129	6	yes		0	0	0	0	0	0	0	0	0	0		
Expt. 334	45	1-25-10	1 cc. virus 124	18	yes		0	0	0	0	0	0	0	0	0	0		
Expt. 394	44	3-27-10	1 cc. virus 158—2 cc. 164	15	yes		—	—	—	—	—	—	—	—	0	0		
Expt. 409	24	5-5-10	2 cc. virus 170	7	yes		0	0	0	0	0	0	0	0	0	0		
Expt. 419	23	6-2-10	1 cc. virus 175	8		yes	0	0	0	0	0	0	0	0	0	0		

Of the 56 samples of virus blood tested,

7 or 12.5 per cent gave no reaction at a dilution of 1—50.
4 or 7 1-7 per cent gave a reaction at a dilution of 1—50.
3 or 5 5-14 per cent gave a reaction at a dilution of 1—100.
6 or 10 5-7 per cent gave a reaction at a dilution of 1—125.
12 or 21 3-7 per cent gave a reaction at a dilution of 1—250.
21 or 37.5 per cent gave a reaction at a dilution of 1—500.
3 or 5 5-14 per cent gave a reaction at a dilution of 1—800.

It is readily apparent that the blood of pigs treated with hog cholera virus acquires agglutinin for B. cholerae suis considerably in excess of that found in normal serum. For, whereas only 6¼ per cent of the samples of normal serum agglutinated at a dilution of 1-250, 64 2-7 per cent of the samples of virus blood agglutinated at a dilution of 1-250 or above.

In studying the influence of body weight* upon the agglutinin content of the blood of hog cholera pigs, we get results similar to those recorded under normal serum.

The average weight of

7 pigs whose blood did not react at 1—50 is 37 lbs.
4 pigs whose blood reacted at 1—50 is 63 lbs.
3 pigs whose blood reacted at 1—100 is 90 lbs.
6 pigs whose blood reacted at 1—125 is 83 lbs.
12 pigs whose blood reacted at 1—250 is 55 lbs.
21 pigs whose blood reacted at 1—500 is 66 lbs.
3 pigs whose blood reacted at 1—800 is 95 lbs.

It must be admitted that the relation between weight and agglutinative power of serum is only suggested by these figures. We do not wish to make any generalization yet.

The next factor to be considered that may influence the agglutinative power of the virus blood is the treatment, in other words, the amount of infective blood injected into the pig. The following summary shows the relation between the quantity of virus injected, the average weight of the pigs treated and the average of the agglutination maxima.

Number of pigs.	Amount of virus injected.	Average weight.	Average agglutination, maximum.
7	1 cc	30 lbs	1-197
2	2 cc	36 lbs	No reaction.
26	5 cc	66 lbs	1-288
6	8 cc	72 lbs	1-362
2	9 cc	61 lbs	1-400
13	10 cc	81 lbs	1-392

*Which is in a general way equivalent to age in the pigs here considered.

This summary shows the tendency toward an increase in agglutinative power of blood from heavier hogs in addition to demonstrating the increased agglutinative power as a result of increased dose of virus.

It might be supposed, with reason, that the source of the virus used for treating the pigs would influence the production of agglutinins in the treated pig. It is a point having little force in this connection, since all the virus used (except that on Expt. 291) had the same source, viz. the "Ames virus." The only difference in these virus depends upon the variation in their passage through pigs and in their age.

In all these cases the sample was secured after the pig died from the disease, or was killed. The number of days the pig lived after injection of the virus seems to bear no constant relation to the agglutinative power of the pig's blood serum. Except in those cases where the pig died, it may be assumed that the pig was killed at the height of the disease. We have no data on the rate of increase of the agglutinin after treatment of individual pigs. We learn very little by comparing the reaction of the blood of pigs that died with those that were killed.

REACTIONS WITH THE BLOOD OF PIGS TREATED WITH "MIXED SERUM."

Table III shows the data connected with the testing of blood from 49 pigs treated simultaneously with hog cholera serum and virus. The important features of this table in addition to the agglutination reactions, are the amounts and sources of the sera and virus injected and the results of the treatment.

As for the agglutination reactions, it will be seen that

6 or 12.25 per cent gave no reaction at a dilution of 1—50.
5 or 10.20 per cent reacted at a dilution of 1—50.
6 or 12.25 per cent reacted at a dilution of 1—100.
6 or 12.25 per cent reacted at a dilution of 1—125.
7 or 14.28 per cent reacted at a dilution of 1—250.
19 or 38.77 per cent reacted at a dilution of 1—500.

These results correspond quite closely to those secured with virus blood. One might be tempted to conclude that the influence of the serum could not be detected in the agglutination reactions. It must be borne in mind that the average weight of the pigs in Table III is only 35 lbs. and also that they received only 1 cc. of virus each. Now we can make a fairer comparison of the agglutinative power of the blood serum of pigs treated with hog cholera virus only, and with hog cholera virus and serum simultaneously. The summary on page 12 shows that 7 pigs receiving 1 cc. virus each and weighing on an average of 30 lbs. reacted at an average dilution of 1-197. The average maximum reaction at which agglutination occurred with the blood of 49 pigs treated with both serum and virus is 1-262.

TABLE III.—*Agglutination tests with Culture "Virus 36" and blood of pigs treated with mixed Sera.*

Dilution of serum.						50.		100.		125.		250.		500.	
No. of pig.	Date sample drawn.	Results of treatment.	Date treated.	Virus used on pig.	Serum used on pig.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Expt. 293	1-25-10	Remained well	11-18-09	1 cc. virus 124	5 cc. M. S. 46	+	—	—	—	—	—	—	—	0	0
Expt. 294	1-25-10	Remained well	11-18-09	1 cc. virus 124	10 cc. M. S. 40	—	—	—	—	—	—	0	0	0	0
Expt. 295	1-25-10	Remained well	11-18-09	1 cc. virus 124	15 cc. M. S. 46	—	—	—	—	0	0	0	0	0	0
Expt. 296	1-25-10	Remained well	11-18-09	1 cc. virus 124	20 cc. M. S. 40	—	—	—	—	—	—	0	0	0	0
Expt. 297	1-25-10	Remained well	11-18-09	1 cc. virus 124	25 cc. M. S. 46	—	—	—	—	—	—	—	—	0	0
Expt. 299	12- 1-09	Pig died in 12 days	11-19-09	1 cc. virus 124	5 cc. M. S. 47	0	0	0	0	0	0	0	0	0	0
Expt. 300	1-21-10	Remained well	11-19-09	1 cc. virus 124	10 cc. M. S. 47	+	—	+	—	—	—	—	—	—	—
Expt. 302	1-21-10	Died 1-21-10, not posted	11-19-09	1 cc. virus 124	20 cc. M. S. 47	—	—	—	—	—	—	—	—	—	—
Expt. 303	1-21-10	Remained well	11-19-09	1 cc. virus 124	25 cc. M. S. 47	+	+	+	—	+	—	—	—	—	—
Expt. 307	1-21-10	Remained well	11-29-09	1 cc. virus 127	15 cc. M. S. 35	+	—	+	—	—	—	—	—	—	—
Expt. 308	1-21-10	Remained well	11-29-09	1 cc. virus 127	20 cc. M. S. 35	+	+	—	—	—	—	—	—	—	—
Expt. 309	1-21-10	Remained well	11-29-09	1 cc. virus 127	25 cc. M. S. 35	+	—	—	—	—	—	0	0	0	0
Expt. 312	1-21-10	Remained well	11-30-09	1 cc. virus 128	10 cc. M. S. 41	—	—	0	0	0	0	0	0	0	0
Expt. 313	1-21-10	Remained well	11-30-09	1 cc. virus 128	15 cc. M. S. 41	—	—	0	0	0	0	0	0	0	0
Expt. 314	1-21-10	Remained well	11-30-09	1 cc. virus 128	20 cc. M. S. 41	+	—	+	—	+	—	—	—	—	—
Expt. 315	1-21-10	Remained well	11-30-09	1 cc. virus 128	25 cc. M. S. 41	+	+	+	+	+	+	—	—	—	—
Expt. 317	1-18-10	Remained well	12-15-09	2 cc. filt. virus 136	10 cc. M. S. 35	0	0	0	0	0	0	0	0	0	0
Expt. 318	1-18-10	Remained well	12-15-09	2 cc. filt. virus 136	15 cc. M. S. 35	+	—	—	—	—	—	—	—	0	0
Expt. 324	1-18-10	Remained well	12-29-09	1 cc. virus 124	20 cc. M. S. 36	+	—	—	—	—	—	0	0	0	0
Expt. 325	1-18-10	Remained well	12-29-09	1 cc. virus 124	20 cc. M. S. 39	+	—	—	—	—	—	—	—	—	—
Expt. 326	1-18-19	Remained well	12-29-09	1 cc. virus 124	20 cc. M. S. 38	+	+	—	—	—	—	—	—	—	—
Expt. 327	1-18-10	Remained well	12-29-08	1 cc. virus 124	20 cc. M. S. 40	+	—	—	—	—	—	—	—	—	—
Expt. 328	1-18-10	Remained well	12-29-19	1 cc. virus 124	20 cc. M. S. 42	+	—	—	—	—	—	—	—	—	—
Expt. 330	1-25-10	Remained well	1- 7-10	1 cc. virus 124	20 cc. M. S. 48	—	—	—	—	—	—	—	—	—	—
Expt. 331	1-25-10	Remained well	1- 7-10	1 cc. virus 124	15 cc. M. S. 48	+	+	+	+	—	—	—	—	—	—
Expt. 333	1-25-10	Remained well	1- 7-10	1 cc. virus 124	25 cc. M. S. 48	+	+	—	—	—	—	—	—	—	—
Expt. 335	1-22-10	Died in 15 days, ulcers	1- 7-10	1 cc. virus 124	5 cc. M. S. 49	—	—	—	—	0	0	0	0	0	0
Expt. 336	1-25-10	Alive but very thin	1- 7-10	1 cc. virus 124	10 cc. M. S. 49	—	—	—	—	0	0	0	0	0	0
Expt. 337	1-25-10	Remained well	1- 7-10	1 cc. virus 124	15 cc. M. S. 49	+	+	+	+	+	—	—	—	—	—

TABLE III.—*Concluded.*

No. of pig.	Date sample drawn.	Results of treatment.	Date treated.	Virus used on pig.	*Serum used on pig.*	Dilution of serum: 50. Agglutination.	50. Sediment.	100. Agglutination.	100. Sediment.	125. Agglutination.	125. Sediment.	250. Agglutination.	250. Sediment.	500. Agglutination.	500. Sediment.
Expt. 338	1-25-10	Remained well	1- 7-10	1 cc. virus 124	20 cc. M. S. 49	—	—	—	—	0	0	0	0	0	0
Expt. 339	1-25-10	Remained well	1- 7-10	1 cc. virus 124	25 cc. M. S. 49	—	—	—	—	0	0	0	0	0	0
Expt. 341	1-30-10	Died in 11 days, acute	1-19-10	1 cc. expt. 340	5 cc. M. S. 25	—	—	—	—	—	—	0	0	0	0
Expt. 345	1-31 10	Died in 12 days, acute	1 19-10	1 cc. expt. 340	10 cc. M. S. 32	—	—	0	0	0	0	0	0	0	0
Expt. 346	1-30-10	Died in 11 days, acute	1-19-10	1 cc. expt. 340	15 cc. M. S. 32	—	—	—	—	—	—	—	—	0	0
Expt. 347	2- 5-10	Died in 17 days, acute	1-19-10	1 cc. expt. 340	5 cc. M. S. 33	—	—	0	0	0	0	0	0	0	0
Expt. 348	2- 8-10	Died in 20 days, ulcers	1-19-10	1 cc. expt. 340	10 cc. M. S. 33	—	—	—	—	0	0	0	0	0	0
Expt. 349	1-30-10	Died in 11 days, ulcers	1-19-10	1 cc. expt. 340	15 cc. M. S. 33	—	—	—	—	—	—	—	—	—	—
Expt. 350	1-29-10	Died in 10 days, ulcers	1-19-10	1 cc. expt. 340	5 cc. M. S. 34	0	0	0	0	0	0	0	0	0	0
Expt. 351	1-31-10	Died in 12 days, ulcers	1-19-10	1 cc. expt. 340	10 cc. M. S. 34	—	—	0	0	0	0	0	0	0	0
Expt. 356	1-28-10	Died in 9 days, ulcers	1-19-10	1 cc. expt. 340	5 cc. M. S. 43	+	+	—	—	—	—	—	—	0	0
Expt. 363	1-25-10	Died in 6 days	1-19-10	1 cc. expt. 340	10 cc. M. S. 45	+	+	—	—	—	—	—	—	0	0
Expt. 365	1-30-10	Died in 9 days	1-21-10	1 cc. virus 122	10 cc. from virus 139	—	—	—	—	—	—	0	0	0	0
Expt. 373	2- 4-10	Died in 10 days	1-25-10	1 cc. virus 125	10 cc. M. S. 51	0	0	0	0	0	0	0	0	0	0
Expt. 374	2- 8-10	Died in 14 days	1-25-10	1 cc. virus 125	15 cc. M. S. 51	0	0	0	0	0	0	0	0	0	0
Expt. 381	2-16-10	Died in 9 days	2- 7-10	1 cc. virus 153	20 cc. M. S. 52	+	—	—	—	—	—	—	—	0	0
Expt. 406	5- 5-10	Died in 7 days, acute	4-28-10	1 cc. virus 170	10 cc. M. S. 56	0	0	0	0	0	0	0	0	0	0
Expt. 396	5- 5-10	Died in 8 days, acute	4-27 10	1 cc. virus 170	15 cc. from expt. 315	—	—	—	—	—	—	—	—	—	—
Expt. 395	5- 5-10	Died in 8 days, acute	4-27-10	1 cc. virus 170	10 cc. from expt. 315	+	—	—	—	—	—	—	—	—	—
Expt. 402	5-20-10	Died in 22 days	4-28-10	1 cc. virus 170	10 cc. M. S. 52	—	—	—	—	—	—	—	—	—	—

It will be of interest to note the difference in the agglutinative power of the blood from the pigs that were protected by the serum and from those that died. We have made this difference very readily perceptible in the following summary.

Dilution of serum.	Lived.		Died.	
	Number of pigs.	Percentage.	Number of pigs.	Percentage.
0	1	3.6	5	23.8
1-50	2	7.1	3	14.3
1-100	4	14.3	2	9.5
1-125	4	14.3	2	9.5
1-250	3	10.7	4	19.1
1-500	14	50	5	23.8

A comparatively much larger number of pigs that die fail to show any agglutinative power in their serum, while the serum of a comparatively greater number of pigs that live gives a reaction at high dilution. In other words, it seems that with resistance or immunity we get increased agglutinative power; with susceptibility or absence of immunity, a less or no increase. This is in keeping with the deduction that heavier or older pigs (which are well known to be more resistant to cholera infection) have blood serum with a higher agglutinin content. We can find no relation between the amount of protective serum injected into the pig and the production of agglutinins in the pig's blood.

REACTIONS WITH THE BLOOD OF HYPERIMMUNIZED PIGS.

This phase of the subject has received considerable attention. It has been our aim to determine the effect of repeated injections of virus blood and tail bleedings on the agglutinative power of the blood of "serum hogs."

We assume that the following factors influence the production of agglutinins in the body fluids:

TABLE IV.—*Showing agglutination reactions with blood from serum hog 136.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
2100 cc	11-24-09	...	+	+	+	+	−	−	−	−	...	...	...	...	...	...	...	...	...	...	...	...
2100 cc	12- 1-09	First	+	+	+	+	+	+	+	+	+	−	...	...	...	...	...	...	...	...	...	...
2100 cc	12- 8-09	*Second*	...	...	...	...	+	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...
2100 cc	12-15-09	Third	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
2400 cc	12-22-09	Fourth	...	...	...	...	...	...	...	...	...	...	...	...	−	−	−	−	−	−	−	−
2700 cc	12-29-09	*Fifth*	...	...	...	...	...	...	...	...	+	+	+	−	+	−	+	−	−	−	−	−
2700 cc	1- 5-09	Killed	...	...	...	...	...	...	...	...	+	+	+	+	+	−	+	−	+	−	−	−
Mixed serum 48	...	...	...	...	...	...	...	...	...	...	+	+	+	−	−	−	−	−	−	−	−	−
Tested.	**Date drawn.**	**Preservative.**																				
2-21-10	12-15-09	.5% phenol	...	...	+	−	+	−	+	−	+	−	−	−	...	...	...	...	...	...	...	...
2-21-10	12-15-09	.5% trilauce	...	...	+	−	+	−	+	−	+	−	−	−	...	...	...	...	...	...	...	...
2-21-10	12-15-09	.2% formol	...	...	+	−	+	−	+	−	+	−	−	−	...	...	...	...	...	...	...	...
2-21-10	12-15-09	.5% fromol	...	...	+	−	+	−	+	−	+	−	−	−	...	...	...	...	...	...	...	...

TABLE V.—*Showing agglutination reactions with blood from serum hog 138.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
800 cc	11-22-09		+	+	+	+	+	+	−	−	−											
1200 cc	11-26-09		+	+	+	−	+	−	−	−	0	0										
1700 cc	12- 3-09										+	−	−	−	−	−	−	−				
2300 cc	12-10-09	First	+	+	+	+	+	+	+	−	−	−										
2300 cc	12-17-09	Second	+	−	+	−	+	−	+	−	−	−										
2900 cc	12-24-09	Third	+	−	+	−	−	−	−	−	−	−										
2900 cc	12-31-09	Fourth	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0	0
3600 cc	1-19-09	Fifth			+	−	+	−	+	−	−	−	−	−								
3600 cc	1-27-10	Killed			+	−	+	−	+	−	−	−	−	−								
Mixed serum 52																						

TABLE VI.—*Showing agglutination reactions with blood from serum hog 137.*

Dilution of serum.			400.		800.		1000.		2000.		4000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
900 cc	11-22-09		—	—	0	0	0	0	0	0	......	......
1300 cc	11-26-09		—	—	0	0	0	0	0	0	0	0
2250 cc	12-10-09	First	—	—	—	—	—	—	—	—	0	0
2250 cc	12-17-09	Second	+	—	—	—	—	—	—	—	—	—
2550 cc	12-24-09	Third	—	—	—	—	—	—	—	—	0	0
2965 cc	12-31-10	Fourth	+	—	+	—	—	—	—	—	—	—
3265 cc	1- 7-10	Fifth	+	—	—	—	—	—	—	—	0	0
3265 cc	1-14-10	Sixth	+	—	—	—	—	—	—	—	0	0
3265 cc	1-21-10	Seventh	+	—	—	—	—	—	0	0	0	0
3265 cc	1-28-10	Killed	+	—	—	—	—	—	—	—	—	—
Mixed serum 53			+	+	—	—	—	—	—	—	—	—

TABLE VII.—*Showing agglutination reactions with blood from serum hog 139.*

Dilution of serum.			400.		800.		1000.		2000.		4000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
2000 cc	11-24-09		—	—	0	0	0	0	0	0	......	......
2000 cc	12- 1-09	First	—	—	—	—	—	—	0	0	......	......
2000 cc	12- 8-09	Second	—	—	0	0	0	0	0	0	......	......
2000 cc	12-15-09	Third	—	—	—	—	—	—	—	—	—	—
2300 cc	12-22-09	Fourth	+	—	—	—	—	—	0	0	0	0
2600 cc	12-29-09	Fifth	—	—	—	—	0	0	0	0	0	0
2600 cc	1- 5-10	Killed	—	—	—	—	—	—	0	0	0	0
Mixed serum 49			+	—	—	—	—	—	—	—	0	0

TABLE VIII.—*Showing agglutination reactions with blood from serum hog 140.*

Dilution of serum.			400.		800.		1000.		2000.		4000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1270 cc	10-16-09	First	......	......	—	—	—	—	—	—	0	0
1420 cc	10-23-09	Second	......	......	—	—	—	—	0	0	0	0
1420 cc	10-30-09	Third	......	......	—	—	—	—	0	0	0	0
1420 cc	11- 6-09	Killed	......	......	—	—	—	—	0	0	0	0
Mixed serum 46			—	—	—	—	0	0	0	0	0	0

TABLE IX.—*Showing agglutination reactions with blood from serum hog 141.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1460 cc......	10-16-09	First...	......	......	+	+	+	+	+	—	—	—	—	—
1860 cc......	10-23-09	Second.	......	......	+	+	+	+	+	+	—	—	—	—
1660 cc......	10-30-09	Third..	......	......	+	+	+	+	+	+	—	—	—	—
1660 cc......	11- 6-09	Killed..	......	......	+	+	+	+	+	+	—	—	0	0
Mixed serum 47.......	....	....	+	+	+	+	—	—	—	—	—	—	......	......

TABLE X.—*Showing agglutination reactions with blood from serum hog 144.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
50 cc......	12-22-09	....	0	0	0	0	0	0	0	0	0	0	......	......
250 cc......	12- 3-09	....	0	0	0	0	0	0	0	0	0	0	......	......
940 cc......	12-17-09	....	+	+	+	+	—	—	—	—	—	—	......	......
1790 cc......	12-30-09	First...	+	+	—	—	—	—	—	—	—	—	......	......
1790 cc......	1- 6-10	Second.	+	+	—	—	—	—	—	—	0	0	......	......
1790 cc......	1-13-10	Third..	+	—	—	—	—	—	—	—	0	0	......	......
1790 cc......	1-20-10	Killed..	+	—	—	—	—	—	—	—	0	0	......	......
Mixed serum 50.......	....	....	+	—	—	—	—	—	—	—	0	0	......	......

TABLE XI.—*Showing agglutination reactions with blood from serum hog 143.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
50 cc......	11-22-09	....	0	0	0	0	0	0	0	0	......	......	......	......
250 cc......	11-26-09	....	0	0	0	0	0	0	0	0	......	......	......	......
1602 cc......	1-14-10	First...	+	+	+	—	+	—	—	—	—	—	......	......
1602 cc......	1-21-10	Second.	+	—	—	—	—	—	—	—	—	—	......	......
1602 cc......	1-28-10	Third..	+	—	—	—	—	—	—	—	—	—	......	......
1602 cc......	2- 4-10	Killed..	—	—	—	—	—	—	—	—	0	0	......	......
Mixed serum 54.......	....	....	+	—	—	—	—	—	—	—	0	0	......	......
Mixed serum 54a......	....	....	+	—	—	—	—	—	—	—	0	0	......	......

TABLE XII.—*Showing agglutination reactions with blood from serum hog 149.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail of other bleeding.	*Agglutination.*	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1425 cc.	4-30-10	Second	+	+	+	+	+	+	+	—	—	—	....	....	....	....	....	....	....	....	....	....
1425 cc.	5- 9-10	Third	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—	—
1625 cc.	5-16-10	Fourth	....	....	....	....	....	....	....	....	....	....	+	—	—	—	—	—	—	—	—	—
1725 cc.	5-23-10	Fifth	....	....	....	....	....	....	....	....	....	....	+	—	—	—	—	—	—	—	0	0
1725 cc.	5-31-10	Killed	....	....	....	....	....	....	....	....	....	....	+	—	—	—	—	—	—	—	0	0
Mixed serum 60	....	....	....	....	....	....	....	....	....	....	....	....	+	—	+	—	+	—	—	—	—	—

TABLE XIII.—*Showing agglutination reactions with blood from serum hog 145.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	*Agglutination.*	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	*Agglutination.*	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
50 cc.	11-22-09	....	—	—	0	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....
250 cc.	12- 3-09	....	—	—	0	0	0	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....
725 cc.	12- 9-09	....	—	—	—	—	—	—	0	0	0	0	....	....	....	....	....	....	....	....	....	....
1175 cc.	12-13-09	....	—	—	—	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....
1750 cc.	12-20-09	First	+	—	+	—	+	—	+	—	—	—	....	....	....	....	....	....	....	....	....	....
1750 cc.	12-27-09	Second	+	+	+	+	+	+	+	—	—	—	....	....	....	....	....	....	....	....	....	....
1750 cc.	1- 3-09	Third	+	—	+	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....
1750 cc.	1-10-10	Fourth	+	—	—	—	—	—	—	—	0	0	....	....	....	....	....	....	....	....	....	....
2050 cc.	1-17-10	Fifth	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2050 cc.	1-24-10	Killed	+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Mixed serum 51	....	....	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0

TABLE XIV.—*Showing agglutination reactions with blood from serum hog 150.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1225 cc........	4-23-10	First........	+	—	—	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....
1425 cc........	4-30-10	Second.......	+	+	+	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....
1425 cc........	5- 9-10	Third........	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	—	+	—	—	—
1625 cc........	5-16-10	Killed.......	....	....	....	....	....	....	....	....	....	....	+	—	+	—	+	—	+	—	—	—
Mixed serum 59..	....	....	....	....	....	....	....	....	....	....	....	....	+	—	+	—	+	—	+	—	+	—

TABLE XV.—*Showing agglutination reactions with blood from serum hog 147.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1830 cc........	3-28-10	First........	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
1830 cc........	4- 4-10	Second.......	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—
1830 cc........	4-11-10	Third........	....	....	....	....	....	....	....	....	....	....	+	+	—	—	—	—	—	—	—	—
1830 cc........	4-18-10	Fourth.......	....	....	....	....	....	....	....	....	....	....	+	+	—	—	—	—	—	—	—	—
2130 cc........	4-25-10	Fifth........	....	....	....	....	....	....	....	....	....	....	+	—	—	—	—	—	—	—	—	—
2430 cc........	5- 2-10	Sixth........	....	....	....	....	....	....	....	....	....	....	+	—	—	—	—	—	—	—	—	—
2430 cc........	5-10-10	Killed.......	....	....	....	....	....	....	....	....	....	....	+	—	+	—	—	—	—	—	—	—
Mixed serum 57..	....	....	....	....	....	....	....	....	....	....	....	....	+	—	+	—	+	—	+	—	—	—

TABLE XVI.—*Showing agglutination reactions with blood from serum hog 146.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
50 cc	11-22-09		+	+	−	−	−	−	−	−												
200 cc	12- 3-09		+	−	+	−	−	−	0	0	0	0										
1725 cc	1-21-10	First	+	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	0	0	0	0
1725 cc	1-28-10	Second	−		+	−	+	−	+	−	−	−	−	−	−	−						
1725 cc	2- 4-10	Third			+	−	−	−	−	−	−	−	−	−	−	−						
1725 cc	2-11-10	Fourth			+	−	+	−	+	−	+	−	−	−	−	−						
2027 cc	2-18-10	Killed			+	−	+	−	+	−	+	−	+	−	−	−						
Mixed serum 55					+	−	+	−	+	−	+	−	+	−	−	−						

TABLE XVII.—*Showing agglutination reactions with blood from serum hog 151.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination	Sediment.
1850 cc	4-30-10	Second	+	−	−	−	−	−	−	−	−	−										
1350 cc	5- 9-10	Third	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
1550 cc	5-16-10	Fourth											−	−	−	−	−	−	−	−	0	0
1850 cc	5-23-10	Fifth											+	−	−	−	−	−	−	−	0	0
1850 cc	5-31-10	Sixth											+	−	−	−	−	−	−	−	0	0
1975 cc	6- 8-10	Killed											+	+	−	−	−	−	−	−	−	−
Mixed serum 61													+	−	−	−	−	−	−	−	−	−

TABLE XVIII.—*Showing agglutination reactions with blood from serum hog 148.*

Dilution of serum.			2000.		4000.		5000.		10000.		12500.		25000.		50000.		100000.		125000.		500000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
2320 cc.	3-22-10	First.	+	+	+	+	+	—	—	—	—	—	—	—	—	—	......	......	......	......	......	......
2320 cc.	3-29-10	Second.	......	......	......	......	+	—	—	—	—	—	—	—	—	—	......	......	......	......	......	......
2320 cc.	4- 5-10	Third.	......	......	......	......	+	—	—	—	—	—	—	—	—	—	......	......	......	......	......	......
2320 cc.	4-12-10	Fourth.	......	......	......	......	+	+	—	—	—	—	—	—	—	—	......	......	......	......	......	......
2320 cc.	4-18-10	Fifth.	......	......	......	......	+	—	—	—	—	—	—	—	—	—	......	......	......	......	......	......
2620 cc.	4-25-10	Sixth.	......	......	......	......	+	—	—	—	—	—	—	—	—	—	......	......	......	......	......	......
2920 cc.	5- 2-10	Seventh.	......	......	......	......	......	......	......	......	......	......	......	......	+	—	—	—	0	0	......	......
2920 cc.	5-10-10	Killed.	......	......	......	......	......	......	......	......	......	......	......	......	+	—	—	—	—	—	0	0
Mixed serum 58.	......	......	......	......	......	......	+	—	+	—	+	—	—	—	—	—	......	......	......	......	......	......

TABLE XIX.—*Showing agglutination reactions with blood from serum hog 152.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		5000.		10000.		12500.		25000.		50000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1250 cc.	4-23-10	First.	+	—	—	—	—	—	—	—	—	—	......	......	......	......	......	......	......	......	......	......
1450 cc.	4-30-10	Second.	+	+	+	+	+	—	—	—	—	—	......	......	......	......	......	......	......	......	......	......
1450 cc.	5- 9-10	Third.	+	+	+	+	+	+	+	—	+	—	+	—	+	—	—	—	—	—	—	—
1650 cc.	5-16-10	Fourth.	......	......	......	......	......	......	......	......	......	......	+	—	—	—	—	—	—	—	—	—
1750 cc.	5-23-10	Fifth.	......	......	......	......	......	......	......	......	......	......	+	—	+	—	—	—	—	—	—	—
1750 cc.	5-31-10	Killed.	......	......	......	......	......	......	......	......	......	......	+	—	—	—	—	—	—	—	—	—
Mixed serum 62.	......	......	......	......	......	......	......	......	......	......	......	......	+	—	+	—	+	—	—	—	—	—

TABLE XX.—*Showing agglutination reactions with blood from serum hog 155.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1115 cc......	5-28-10	First...	+	−	−	−	−	−	−	−	−	−	......	......
1115 cc......	6- 4-10	Second.	+	+	+	+	+	+	−	−	−	−	......	......
1295 cc......	6-11-10	Third..	+	+	+	+	+	+	−	−	−	−	......	......
1295 cc......	6-18-10	Killed..	......	......	+	−	−	−	−	−	−	−	0	0
Mixed serum 63........	....	....	+	+	+	+	+	−	+	−	−	−	......	......

TABLE XXI.—*Showing agglutination reactions with blood from serum hog 157.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1490 cc......	6- 2-10	First...	+	−	+	−	+	−	−	−	−	−	......	......
1490 cc......	6- 9-10	Second.	+	+	+	+	+	+	+	+	−	−	......	......
1490 cc......	6-16-10	Third..	+	+	+	+	+	+	−	−	−	−	......	......
1690 cc......	6-23-10	Killed..	......	......	+	−	−	−	−	−	−	−	0	0
Mixed serum 64........	....	....	+	+	+	−	+	−	−	−	−	−	......	......

TABLE XXII.—*Showing agglutination reactions with blood from serum hog 158.*

Dilution of serum.			400.		800.		1000.		2000.		4000.		8000.	
Amount of virus injected into pig.	Date of drawing sample.	Tail or other bleeding.	Agglutination.	Sediment.	Agglutination	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
1325 cc......	6- 2-10	First...	+	−	+	−	+	−	+	−	−	−	......	......
1325 cc......	6- 9-10	Second.	+	+	+	+	+	+	+	+	−	−	......	......
1325 cc......	6-16-10	Third..	+	+	+	+	+	+	+	+	−	−	......	......
1525 cc......	6-23-10	Killed..	+	−	+	−	−	−	−	−	−	−	......	......
Mixed serum 65........	....	....	+	+	+	+	+	−	+	−	−	−	......	......

1. The nature of the substance or agglutinogen introduced into the body.
2. The amount of agglutinogen introduced.
3. The number of injections and the length of time required to introduce the total amount of agglutinogen.
4. The method of introducing the agglutinogen.
5. Idiosyncrasy or individual variation in the body receiving the agglutinogen.

There may be many other influencing factors. The agglutination titer of any sample of blood serum depends to a great extent upon the time it is drawn relative to the time of treatment of the animal. Other factors influence the agglutinative power of a sample of serum after it is drawn, such as age, temperature and light. These can be easily controlled.

Of the factors above enumerated which influence the production of agglutinins in the body the first four are subject to control, the individual variation in animals only, being beyond our control. As an illustration of this last point we may say that if we take two pigs of the same sex and litter and as nearly alike as conceivable and subject these pigs to identical treatment in so far as factors 1, 2, 3, and 4 are concerned, we may not get identical results when we test samples of their blood for agglutinative power.

The 19 pigs furnishing the basis of the work outlined in Tables IV to XXII varied greatly in weight, breeding and other characteristics. We could not expect them to respond identically to identical treatment even were there no question of idiosyncrasy. As a matter of fact, the treatment of these pigs has been variable both as regards factors, 1, 2, 3 and 4 (considered above) and as regards withdrawal of blood for agglutination tests or other purposes. A comparison of the results secured from a study of Tables IV to XXIII is, therefore, very difficult.

There is a considerable variation in the maximum dilutions at which agglutination occurred.

Of the 19 cases studied,

1 or 5.26 per cent gave a maximum reaction at 1—2,000.
7 or 36.85 per cent gave a maximum reaction at 1—4,000.
1 or 5.26 per cent gave a maximum reaction at 1—8,000.
2 or 10.52 per cent gave a maximum reaction at 1—12,500.
1 or 5.26 per cent gave a maximum reaction at 1—25,000.
7 or 36.85 per cent gave a reaction at 1—50,000.

It is apparent that a little less than half of the maximum reactions are at one extreme, 200 to 4,000; and a little less than half at the other extreme, 25,000 to 50,000, with a few cases between these extremes.

A study of one case will illustrate what might be called a typical response to several influencing factors, such as increasing the total of virus injected and repeated bleedings.

Serum hog 145 (Table XIII) wt. 56 lbs., July 17, 1909, received 30 cc. mixed serum, 30, and 1 cc. virus 107. The weight increased to 150 lbs. during the following treatment:

Date of test.	Amount of virus injected to date.	Maximum dilution at which agglutination occurred.	Bleeding.
11-22-09	50 cc.	1-400	5 cc. from tail.
12- 3-09	250 cc.	1-400	5 cc. from tail.
12- 9-09	725 cc.	1-1000	5 cc. from tail.
12-13-09	1175 cc.	1-4000	5 cc. from tail.
12-20-09	1175 cc.	1-4000	First tail.
12-27-09	1750 cc.	1-4000	Second tail.
1- 3-10	1750 cc.	1-4000	Third tail.
1-10-10	1750 cc.	1-2000	Fourth tail.
1-17-10	2050 cc.	1-50,000	Fifth tail.
1-24-10	2050 cc.	1-25,000	Killed.
1-30-10	2050 cc.	1-12,500	Mixed 1—6.

A total injection of 50 cc. caused a reaction at 1-400; an increase of 200 cc. produces no change. A total of 725 cc. gives a reaction at 1-1,000, while a total of 1175 cc. results in a reaction at 1-4000 which is maintained until the first bleeding. An increase of 575 cc. produces no change in the agglutinative reaction for the second and third bleedings and results in a fall to 1-2000 at the fourth bleeding. Another injection of 300 cc. brings the reaction up to 1-50,000 but at the last bleeding it falls to 1-25,000. The reaction produced by a mixture of the different bleedings is about an average of the reactions for all.

The increase in virus injected seems to result in an increase (not necessarily *pari passu*) in the agglutinin content of the serum. The withdrawal of a considerable amount of blood tends to produce a decrease in the agglutinin content which may or may not be offset by virus injections. It is true that the response is very irregular as can be seen by studying the tables.

It is not maintained that it is the blood injected or the ultravisible virus that stimulates the production of agglutinins but the *B. cholerae suis* in the blood. In other words we assume that it is the number of *B. cholerae suis* in a quantity of virus and not the volume that affects the agglutinin production in the treated pig.

Unfortunately we were not in a position to make bacterial counts of any great number of samples of virus. We have not found above 10,000 bacteria per cc. in fresh virus. If all these organisms were *B. cholerae suis* the total number in 3,000 cc. of virus would be 30,000,000. We have estimated (by a number of platings) that 1 cc. of a 24 hour bouillon culture of *B. cholerae suis* contains about 500,000,000 bacteria. We have not used so much as 3,000 cc. of virus on any of the 19 cases studied, therefore we have probably not injected more *B. cholerae suis* than would be found in .10 cc. of a 24 hour bouillon culture.

In one case we found that the injection of 10 cc. of a 24 hour bouillon culture of *B. cholerae suis* "virus 136" resulted in a serum giving a

maximum reaction at 1-500. In another case after an injection of 450 cc. of bouillon cultures 15 to 20 hours old mixed with 915 cc. normal pig blood an agglutination reaction was secured at a maximum of 1-25,000. In this case we probably introduced approximately 225 billions of living *B. cholerae suis* or about 7,500 times as many as injected into any of the seven cases, (in Tables IV to XXIII) that reacted at 1-50,000.

It is our belief that the number of *B. cholerae suis* increases greatly locally in the tissues of the injected pig, thus furnishing the agglutinogen in great amount. This belief is strengthened by the almost constant presence of the so-called *B. cholerae suis* abscesses in serum hogs treated intramuscularly.

AGGLUTINATION REACTIONS WITH MIXED SERA.

The term "mixed serum" in this paper is applied to a mixture of all the bleedings from one serum hog. Table XXIII shows the agglutination reactions with 51 samples of mixed sera. These tests were made at a variable length of time after drawing the blood and mixing it. Table XXIV shows retests with 25 of these samples and with a mixture of several others (m. s. 56).

Excepting the mixed sera and the four samples in Table IV, all the tests have been made with fresh serum separated from the corpuscles in defibrinated blood, without the addition of a preservative. All the samples in Tables XXIII and XXIV were defibrinated blood preserved in .5 per cent. phenol (10 cc. 5 per cent. phenol to 90 cc. defibrinated blood) except mixed serum 54a which was preserved by the addition of .5 cc. trikresol to 99.5 cc. defibrinated blood. The mixed sera were kept in cold storage at 6°-15° C.

Table XXIII shows that out of 51 mixed sera tested,

3 or 5.88 per cent did not agglutinate at 1—400.
3 or 5.88 per cent agglutinated at 1—400.
3 or 5.88 per cent agglutinated at 1—800.
2 or 3.92 per cent agglutinated at 1—1,000.
10 or 19.60 per cent agglutinated at 1—2,000.
12 or 23.52 per cent agglutinated at 1—4,000.
1 or 1.96 per cent agglutinated at 1—5,000.
4 or 7.84 per cent agglutinated at 1—8,000.
6 or 11.76 per cent agglutinated at 1—12,000.
7 or 13.72 per cent agglutinated at 1—50,000.

The object in the retests was to determine to what extent diminution in agglutinins or agglutinative power takes place with age. It was thought that perhaps we might be able to throw some light upon the subject of diminution or weakening of antibodies in general, including the protective substances against hog cholera. We do not possess the records of any extensive biological tests that show how long the Dorset-Niles serum retains its potency.

TABLE XXIII.—*Showing agglutination reactions with mixed sera and culture "Virus 136."*

Dilution of serum.			400.		800.		1000.		1600.		2000.		3200.		4000.		5000.		8000.		10000.		12000.		12500.		25000.		50000.	
No. of serum.	Date of test.	Approximate age of serum.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Mixed serum 15..	11-10-09	1 year........	+	+	—	—	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 16..	11-10-09	1 year........	0	0	0	0	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 17..	11-10-09	11 months......	+	+	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 18..	11-10-09	10 months......	+	+	+	—	—	—	....	....	—	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 19..	11-10-09	8 months......	—	—	0	0	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 20..	12- 1-09	9 months......	—	—	0	0	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 21..	12- 6-09	9 months......	+	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 22..	12- 1-09	9 months......	0	0	0	0	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 23..	12- 1-09	7 months......	+	+	+	—	+	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 24..	12- 1-09	7 months......	+	+	+	+	+	+	....	....	+	+	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 25..	12-1 -09	7 months......	+	+	+	+	+	+	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 26..	12- 6-09	5 months......	+	+	+	—	+	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 27..	12- 6-09	5 months......	+	+	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 28..	12- 6-09	5 months......	—	—	—	—	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 29..	12- 6-09	5 months......	—	—	—	—	—	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 30..	12- 2-09	5 months......	0	0	0	0	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 31..	12- 2-09	5 months......	+	—	0	0	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 32..	12- 2-09	5 months......	+	+	+	+	—	—	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 33..	12- 6-09	5 months......	+	+	+	+	+	+	....	....	+	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 34..	12- 6-09	5 months......	—	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 35..	12- 0-09	1 month......	+	+	+	+	+	+	....	....	+	+	....	....	+	+	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 36..	12-24-09	2 months......	+	+	—	—	—	—	....	....	—	—	....	....	0	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 37..	12-29-09	2 months......	+	+	+	+	+	+	+	—	+	—	+	—	+	—	—	—	—	—	....	....	—	—	....	....	....	....	....	....
Mixed serum 38..	12-29-09	2 months......	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	....	....	0	0	....	....	....	....	....	....
Mixed serum 39..	12-24-09	2 months......	+	—	—	—	—	—	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 40..	12-29-09	2 months......	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	....	....	0	0	....	....	....	....	....	....
Mixed serum 41..	11-22-09	2 months......	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 42..	12-29-09	2 months......	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	....	....	—	—	....	....	....	....	....	....
Mixed serum 43..	12-29-09	2 months......	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	....	....	—	—	....	....	....	....	....	....
Mixed serum 44..	12-29-09	2 months......	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	—	....	....	+	—	....	....	....	....	....	....

TABLE XXIII.—*Concluded.*

Dilution of serum.			400.		800.		1000.		1600.		2000.		3200.		4000.		5000.		8000.		10000.		12000.		12500.		25000.		50000.	
No. of serum.	Date of test.	Approximate age of serum.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Mixed serum 45	12- 9-09	2 months	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—			—	—						
Mixed serum 46	11-22-09	Few days	—	—	—	—	0	0	....	....	0	0	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 47	11-22 09	Few days	+	+	+	+	+	+	....	....	+	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 48	1 12-10	Few days	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	—	—	....	....	—	—	—	—	—	—
Mixed serum 49	1-12-10	Few days	+	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 50	1-31-10	Few days	+	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 51	1-29 10	Few days	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	....	....	—	—	....	....	....	....	....	....
Mixed serum 52	1-29-10	Few days	....	....	+	—	....	....	+	—	—	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....
Mixed serum 53	1-29 10	Few days	+	+	—	—	—	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 54	2-13-10	Few days	+	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 54a	2-13-10	Few days	+	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 55	3- 7-10	Few days	....	....	+	+	....	....	+	—	+	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....
Mixed serum 57	7-22-10	2 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	+	—	....	....	+	—	—	—	—	—
Mixed serum 58	7-22 10	2 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	+	—	....	....	+	—	—	—	—	—
Mixed serum 59	7-22-10	2 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	+	—	....	....	+	—	+	—	+	—
Mixed serum 60	8-10-10	1 month	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	+	—	....	....	+	—	—	—	—	—
Mixed serum 61	8-10 10	1 month	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	—	—	....	....	—	—	—	—	—	—
Mixed serum 62	8-10 10	1 month	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—	....	....	+	—	....	....	+	—	—	—	—	—
Mixed serum 63	8-10-10	1 month	+	+	+	+	+	—	....	....	+	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 64	8-10-10	1 month	+	+	+	—	+	—	....	....	—	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 65	8-10-10	1 month	+	—	+	—	+	—	....	....	+	—	....	....	—	—	....	....	....	....	....	....	....	....	....	....	....	....	....	....

In Table XXV there are found in parallel columns the results of the tests and subsequent retests of 25 mixed sera. Tables XXIII and XXIV show the dates of the respective tests and retests. It is seen that the retests were made 6-8 months after the first tests.

The retests show

In 7 cases no change in agglutinative power.
In 4 cases a 25 per cent decrease.
In 3 cases a 50 per cent decrease.
In 1 case a 68 per cent decrease.
In 5 cases a 75 per cent decrease.
In 1 case an 80 per cent decrease.
In 4 cases an apparent increase.

Any increase is probably only apparent. In case of mixed serum 30 the first test failed to establish a maximum showing only that it was less than 400. The first test with mixed serum 31 probably did not establish a maximum since no test was made between 400 and 800. The same is true with mixed sera 37 and 44 when no attempt was made to get a reaction above 1-12,000.

We have not found that the presence of carbolic acid, trikresol or formalin has any depressing effect upon either the agglutinative power or potency of hog cholera serum.

AGGLUTINATIVE POWER AND POTENCY.

The question as to whether we can turn the agglutination test to account in measuring the potency of serum is answered in Table XXV. It must be confessed that the answer is not very definite. Table XXV shows the agglutinative power of the sera and the results of the biological tests. There are given the different amounts of serum used, the weight of the pigs employed and the results. In all cases the serum was tested against 1 cc. of virulent hog cholera blood. The virulence of the blood was always determined by the use of check pigs corresponding in weight, thrift and origin to the test pigs.

It would be possible to pick out a number of cases of mixed sera that showed low agglutinative power and low potency, and it would be easy to pick out cases showing a high agglutinative power and high potency. If we could arrange all the cases under these two headings the matter would be answered very satisfactorily but we can find cases of low agglutinative power and high potency and those showing high agglutinative power and low potency.

TABLE XXIV.—*Showing Agglutination retests*

Dilution of serum.			50.		100.		125.		200.		250.		400.		500.		800.	
No. of serum.	Date of test.	Approximate age of serum.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
Mixed serum 15	7-17-10	1 yr., 8 mos.	....	....	....	....	....	....	—	—	....	....	0	0	0	0	....	....
Mixed serum 17	7-17-10	1 yr., 7 mos.	....	....	....	....	....	....	—	—	....	....	—	—	—	—	....	....
Mixed serum 18	7-17-10	1 yr., 6 mos.	....	....	....	....	....	....	—	—	....	....	—	—	—	—	....	....
Mixed serum 21	7-17-10	1 yr., 4 mos.	....	....	....	....	....	....	....	....	....	....	+	—	....	....	—	—
Mixed serum 23	7-17-10	1 yr., 2 mos.	....	....	....	....	....	....	....	....	....	....	—	—	....	....	—	—
Mixed serum 30	7-17-10	1 year	—	—	—	—	—	—	....	....	—	—	....	....	0	0	....	....
Mixed serum 31	7-17-10	1 year	—	—	—	—	—	—	....	....	—	—	....	....	—	—	....	....
Mixed serum 36	7-17-10	9 months	....	....	....	....	....	....	+	—	....	....	—	—	—	—	....	....
Mixed serum 38	7-17-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—
Mixed serum 39	7-17-10	9 months	....	....	....	....	....	....	+	—	....	....	—	—	—	—	....	....
Mixed serum 40	7-17-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—
Mixed serum 42	7-17-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—
Mixed serum 43	7-17-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—
Mixed serum 37	7-22-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 44	7-22-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 45	7-22-10	9 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 48	7-22-10	6 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 49	7-22-10	6 months	....	....	....	....	....	....	....	....	....	....	—	—	....	....	—	—
Mixed serum 50	7-22-10	6 months	....	....	....	....	....	....	+	+	....	....	—	—	—	—	....	....
Mixed serum 51	7-22-10	6 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Mixed serum 52	7-22-10	6 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—
Mixed serum 53	7-22-10	6 months	....	....	....	....	....	....	....	....	....	....	—	—	....	....	—	—
Mixed serum 55	7-22-10	4 months	....	....	....	....	....	....	....	....	....	....	....	....	....	....	+	—
Mixed serum 56*	7-22-10	1 year+	....	....	....	....	....	....	—	—	....	....	—	—	—	—	....	....
Mixed serum 54	8-10-10	6 months	....	....	....	....	....	....	+	—	....	....	—	—	—	—	....	....
Mixed serum 54a	8-10-10	6 months	....	....	....	....	....	....	—	—	....	....	—	—	—	—	....	....

*M. S. 56 is a mixture of m. sera 25, 32, 33 and 34 q. v. in table.

with mixed sera and culture "virus 186".

1000.		1600.		2000.		3200.		4000.		5000.		8000.		10000.		12500.		16000.		25000.		50000.	
Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.	Agglutination.	Sediment.
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TABLE XXV.—*Amount of serum required to protect pig against 1 cc. virus.*

Mixed serum.	Maximum dilution at which agglutination occurred. First test.	Maximum dilution at which agglutination occurred. Second test.	Weight of pig.	5 cc. serum.	Weight of pig.	10 cc. serum.	Weight of pig.	15 cc. serum.	Weight of pig.	20 cc. serum.	Weight of pig.	25 cc. serum.	Weight of pig.	30 cc. serum.
15	800	200	15	Died	20	Died	20	Died	20	Died			20	Lived.
16	−400		15	Died	18	Died	20	Died	20	Died			20	Died.
17	2000	500	20	Died	20	Died	20	Lived	25	Lived			25	Lived.
18	8000	500			56	Died	60	Lived	60	Lived			65	Lived.
19	400				30	Died	30	Died	37	Lived	38	Died		
20	400				35	Died	39	Died	42	Died	43	Died		
21	2000	1000			28	Lived	27	Lived	30	Lived				
22	−400				45	Died	20	Died	20	Died	20	Died		
23	4000	800	26	Lived	24	Lived	24	Lived						
24	4000		24	Lived	22	Lived	22	Lived						
25	4000		13	Died	19	Died in 68 days	55	Died—not h. c.						
26	4000				24	Lived	23	Lived	26	Lived	18	Lived		
27	2000				22	Lived	26	Died	20	Lived	20	Lived		
28	800				23	Lived	27	Lived	21	Lived	27	Died in 43 days		
29	4000				38	Lived	46	Lived	32	Lived	26	Lived		
30	−400	250			25	Lived	34	Died	26	Lived	45	Lived		
31	400	500			27	Died	28	Lived	48	Lived	37	Lived		
32	1000		20	Died	18	Died	28	Died						
33	4000		23	Died, ascarides	20	Died, chronic	42	Died, ascarides						
34	2000		20	Died	21	Died	23	Died						
35	4000		49	Died	49	Died	48	Lived	44	Lived	44	Lived		
36	2000	2000							63	Lived				
37	12000	16000	16	Died	22	Lived	30	Lived						
38	8000	8000							53	Lived				
39	1000	1000							55	Lived				
40	8000	4000							51	Lived				
41	5000		55	Died	44	Lived	46	Lived	50	Lived	37	Lived		
42	12000	8000									48	Lived		
43	12000	8000	13	Died, chronic	19	Died in 63 days	26	Lived						
44	12000	16000	18	Died, chronic	35	Lived	36	Lived						

Mixed serum.	Maximum dilution at which agglutination occurred. First test.	Maximum dilution at which agglutination occurred. Second test.	Weight of pig.	5 cc. serum.	Weight of pig.	10 cc. serum.	Weight of pig.	15 cc. serum.	Weight of pig.	20 cc. serum.	Weight of pig.	25 cc. serum.	Weight of pig.	30 cc. serum.
45	12000	8000	16	Died, ascarides	19	Died, ascarides	20	Lived						
46	400		36	Lived	33	Lived	33	Lived	27	Lived	26	Lived		
47	4000		35	Died	42	Lived	35	Lived	32	Lived	28	Lived		
48	50000	16000	49	Died	54	Lived	55	Lived	72	Lived	50	Lived		
49	2000	800	34	Died	62	Lived	61	Lived	57	Lived	62	Lived		
50	2000	2000	33	Lived	34	Lived	37	Lived						
51	12000	8000			23	Died in 30 days	18	Died	17	Died				
52	8000	8000	12	Died, ascarides	18	Lived	18	Lived	15	Died, ascarides				
53	4000	1000	15	Died	16	Lived	19	Lived	16	Lived				
54	2000	2000			17	Lived	23	Lived	17	Died, chronic				
54a	2000	2000			28	Died, chronic	28	Lived	29	Lived				
55	8000	4000	38	Died	34	Lived	45	Lived	41	Lived	44	Lived		
56		2000			18	Died	21	Died	20	Died				
57	50000				22	Died	22	Lived	20	Lived				
58	50000				17	Lived	17	Lived	18	Lived				
59	50000				15	Lived	16	Lived	17	Lived				
60	50000				41	Lived	38	Lived						

The following are cases in point:

LOW AGGLUTINATIVE POWER, LOW POTENCY.

Serum.	Agglutinative power.	Potency.
Mixed serum 16	Below 1-400	30 cc. did not protect.
Mixed serum 19	1-400	25 cc. did not protect.
Mixed serum 20	1-400	25 cc. did not protect.
Mixed serum 22	Below 1-400	25 cc. did not protect.

HIGH AGGLUTINATIVE POWER, HIGH POTENCY.

Serum.	Agglutinative power.	Potency.
Mixed serum 48	1-50,000	10 cc. protects.
Mixed serum 57	1-50,000	15 cc. protects.
Mixed serum 58	1-50,000	10 cc. protects.
Mixed serum 59	1-50,000	10 cc. protects.
Mixed serum 60	1-50,000	10 cc. protects.

A conclusion based upon the above data would be misleading in that it would affirm the value of the agglutination test in determining the potency of the Dorset-Niles serum.

The following cases illustrate the possibility of error in estimating potency by agglutinative power:

LOW AGGLUTINATIVE POWER, HIGH POTENCY.

Serum.	Agglutinative power.	Potency.
Mixed serum 28	1-800	10 cc. protects.
Mixed serum 30	*Below 1-400*	*20 cc. protects.*
Mixed serum 31	1-500	15 cc. protects.
Mixed serum 46	1-400	5 cc. protects.

HIGH AGGLUTINATIVE POWER, LOW POTENCY.

Serum.	Agglutinative power.	Potency.
Mixed serum 25	1-4000	15 cc. did not protect.
Mixed serum 32	1-4000	15 cc. did not protect.
Mixed serum 34	1-2000	15 cc. did not protect.
Mixed serum 51	1-12000	20 cc. did not protect.
Mixed serum 56	1-8000	20 cc. did not protect.

On the whole, however, it is to be observed that a serum having a high agglutinative power can be expected to have a higher potency than a serum with low agglutinative power. By potency we mean that the serum was able to protect a small pig against 1 cc. of virulent hog cholera blood in doses not larger than usually recommended by those engaged in serum production.

It may be well to arrange the mixed sera according to their agglutinative power and potency.

Of those agglutinating at

1—50,000........	5 or 100 per cent were potent.
1—12,000........	5 or 83⅓ per cent were potent.
	1 or 16⅔ per cent was not potent.
1—8,000........	4 or 100 per cent were potent.
1—5,000........	1 or -100 per cent was potent.
1—4,000........	7 or 77 7-9 per cent were potent.
	2 or 22 2-9 per cent were not potent.
1—2,000........	8 or 80 per cent were potent.
	2 or 20 per cent were not potent.
1—1,000........	1 or 50 per cent was potent.
	1 or 50 per cent was not potent.
1—800	1 or 50 per cent was potent.
	1 or 50 per cent was not potent.
1—400	2 or 50 per cent were potent.
	2 or 50 per cent were not potent.
Less than.......	1 or 33 1-3 per cent was potent.
1—400	2 or 66 2-3 per cent were not potent.

Of all the mixed sera agglutinating at a dilution of 1-2,000 or above

85.71 per cent were potent.
14.28 per cent were not potent.

Of all the mixed sera agglutinating at a dilution of 1-1,000 or less,

45.45 per cent were potent.
54.54 per cent were not potent.

A very potent serum, such as mixed serum 46 requires only 5 cc. to protect a 36 lb. pig, yet agglutinates at only 1-400. Mixed serum 57 agglutinating at 1-50,000 requires that 15 cc. shall be injected to protect a 22 lb. pig.

After studying the matter very carefully, we have come to the conclusion that the biological test (using small pigs) is very uncertain. Our experience shows that potency may be indicated if pigs weighing 75 to 100 lbs. be used, while lack of potency may be indicated by the use of pigs just weaned. The involvement of ascarides, lung worms and unfavorable climatic or dietary conditions tend to make serum-testing on small pigs very unsatisfactory.

Potency as indicated by our biological tests is only a relative and more or less uncertain matter. Therefore, when we try to compare the results of an easily controlled in vitro test such as the agglutination reaction with a very elastic standard of in vivo testing confusion is sure to ensue. Moreover, we have to admit at the outset that we are dealing with an unknown quantity when we try to introduce *B. cholerae suis* into the subject of potency of serum. We have not yet found an answer to the all important question: "What has *B. cholerae suis* to do with

hog cholera?" Our work furnishes an answer that should serve to keep the subject still in the field for discussion. *B. cholerae suis* has *a great deal* to do with hog cholera and serum production but *what* and *how important* its connection is we cannot say.

Dammann and Stedefeder[4] have recently published the results of a series of researches carried on at the veterinary college at Hanover. They produced hog cholera with filtrates of blood and diseased organs of hog cholera pigs. From one outbreak they isolated an organism showing the morphological and most of the cultural and biological characters of *B. cholerae suis*. This organism they designated *B. suipestifer*, Voldagsen. They were not able to produce hog cholera by use of filtrates from pigs in this outbreak. Cultures of *B. suipestifer*, Voldagsen, fed in large doses produced a septicaemia and death in a few days; fed in small doses it produced a more marked diphtheritic enteritis. The culture injected could be recovered in all cases. Fatal results followed subcutaneous or intravenous injection of their *B. suipestifer*. They likewise succeeded in producing hog cholera (or a disease resembling it) by cohabitation of healthy swine with pigs infected artificially with *B. suipestifer*, Voldagsen, or with pigs naturally infected in the Voldagsen outbreak.

Their agglutination tests and biological tests indicate that *B. suipestifer*, Uhlenhuth, is closely related to *B. paratyphi B*, and that *B. suipestifer*, Voldagsen, is a distinct specific organism having high virulence and producing an epizootic swine disease answering to the descriptions of hog cholera.

They believe that there is sufficient difference in the pathology of the lesions to permit of distinguishing bacillary hog cholera from that produced by the ultravisible virus.

SUMMARY.

GENERAL.

B. cholerae suis is an easily isolated organism in a great many cases of hog cholera. Possibly it is present in all cases.

A living virus capable of producing hog cholera passes through the Chamberland filters that keep back any form of *B. cholerae suis* or other organisms capable of multiplying in vitro to the extent of being susceptible of demonstration.

B. cholerae suis is capable of producing a disease in pigs quite similar to natural hog cholera and to the disease produced by the filterable virus.

The protection offered by the Dorset-Niles serum against the filterable virus may also extend to virulent cultures of *B. cholerae suis*. Whether it is necessary to protect against *B. cholerae suis* in practice is not determined.

The relation of *B. cholerae suis* to the filterable virus or to natural outbreaks of hog cholera is not determined by our work or to our satisfaction by the researches of othe s.

AGGLUTINATION.

The blood of normal (untreated pigs) may agglutinate virulent cultures of *B. cholerae suis* in dilutions as high as 1-250, usually less. The blood of young pigs contains less agglutinin as a rule than that of old pigs.

The blood of pigs having hog cholera as a result of virus inoculation may agglutinate *B. cholerae suis* in dilution as high as 1-800 but usually at a less dilution. Here again age is a factor in that old pigs develop more agglutinin than young ones. (Old pigs are likewise more resistant to hog cholera infection.)

The blood of pigs treated by the serum-simultaneous method may agglutinate *B. cholerae suis* in dilutions as high as 1-500.

The agglutination reaction seems to be one of immunity not of infection, at least, agglutinins develop in connection with immunity but perhaps not as a factor in the condition of immunity. This deduction is based upon the observation that a large percentage of pigs treated by the serum-simultaneous method show a low agglutinative power in the event of death, while of those that live 50 per cent. show the highest agglutinative power.

During the process of hyperimmunization, the agglutinin content of a pig's serum increases as a rule as the amount injected increases, and may fall during the tail bleedings unless more virus be injected.

If the agglutinogen in the virus is *B. cholerae suis* then the quantity of agglutinogen (number of *B. cholerae suis*) injected into a large serum hog during the whole process of hyperimmunization would ordinarily (if only freshly drawn virus is used) be less than would be contained in .10 cc. of a 24-hour bouillon culture of *B. cholerae suis.*

The injection of a number of *B. cholerae suis*, in bouillon culture, equal to that found in the total quantity of virus sufficient to hyperimmunize a large pig fails to stimulate the production of agglutinin to such an extent as is the case when the virus is injected.

Over one-third of the cases of serum hogs studied furnish a serum agglutinating at a dilution of 1-50,000.

The Dorset-Niles serum retains its agglutinative power for several days, almost unimpaired, when preserved in .5 per cent. carbolic acid, trikresol or formalin. The agglutinative power of a serum may diminish 50 per cent., more or less, after a period of 6 to 8 months.

The potency of the Dorset-Niles serum, the biological test being the standard, cannot be measured uniformly by its agglutinative power for *B. cholerae suis*. However, the biological test with pigs is a variable standard.

Sera of high agglutinative power, i. e., reacting at 1-2,000 or above, were potent in 85.71 per cent. of cases and not potent in 14.28 per cent.; sera of low agglutinative power, i. e., reacting at 1-1,000 or less, were potent in 45.45 per cent. of cases and not potent in 54.54 per cent.

The agglutinability of the different cultures used by us indicates that they belong to the same strain. They were isolated from the spleen

Note.—The maximum agglutination titer for pigs treated by the serum-simultaneous method is less than for pigs treated with virus only. This is because the former were all young pigs while the latter were mostly old. Comparing pigs of nearly equal size, those receiving 1 c. c. of virus only showed an average agglutinative power of 1-197, while those receiving 1 c. c. of virus and the Dorset-Niles serum showed an average agglutinative power of 1-262.

of virus pigs treated by virus having a common origin. We therefore believe that these cultures originated, in the original virus and not in an alleged normal habitat in the pig's intestine.

CONCLUSION.

We believe that the relation of *B. cholerae suis* to the porcine organism and to the filterable virus, and all the interrelation of these three factors in the production of a swine disease should be settled. A scientific understanding of hog cholera is impossible without this solution. The economic problems involved in the production of the Dorset-Niles serum or any other biological therapeutic agent for hog cholera, and the sanitary police control and eradication of this disease demand it.

The writer is not interested in a controversy over the merits of the different claims as to the etiologic significance of a non-cultivable, virus or a microscopic, cultivable virus for hog cholera. It is only wished that the matter may be placed beyond the stage of controversy.

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